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## Cardiac Channelopathies: Disease at the Tip of a Patch Electrode

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### 1. Introduction

The ordered electrical excitation of the heart via the cardiac conduction system coordinates the efficient pumping of blood. Electrical impulses normally originate in the sinoatrial node and then propagate through the atria, atrioventricular node, and into the ventricles. The electrical activity of the heart can be measured non-invasively using an electrocardiogram (ECG). An ECG reflects the summed electrical activity of the individual contractile myocytes in the different regions of the heart over time. The first event is the “P wave” and corresponds to the depolarization of the atria, the next three waves “Q, R, and S” represent the progressive wave of depolarization through the ventricles, and the final wave of the cardiac cycle “T” corresponds to ventricular repolarization. The action potentials of contractile atrial and ventricular myocytes share several common characteristics: a stable resting or diastolic membrane potential (phase 4), a rapid depolarization (phase 0), an initial repolarization (phase 1), a plateau (phase 2), and a rapid repolarization to diastolic potential (phase 3). The different phases of the cardiac action potential reflect changes of the sarcolemma permeability to  $K^+$ ,  $Na^+$ , and  $Ca^{2+}$ . Figure 1 shows the relationship between the ECG, a ventricular action potential, and several of the important ventricular  $K^+$ ,  $Na^+$ , and  $Ca^{2+}$  currents (Nerbonne and Kass, 2005).

Arrhythmias are electrical disturbances that disrupt the normal initiation or propagation of the cardiac impulse. They cause abnormal impulse rates (bradycardia or tachycardia), block impulse propagation, or initiate the impulse to circle in a “reentry” loop. Atrial arrhythmias can result in the formation of blood clots and increase the risk of stroke, whereas ventricular arrhythmias can cause the inefficient pumping of blood, loss of consciousness, and sometimes death (Shah et al., 2006).

“Cardiac channelopathies” are caused by mutations in ion channel genes that encode the proteins that underlie the  $K^+$ ,  $Na^+$ , or  $Ca^{2+}$  currents that shape the cardiac action potential (Figure 1). These mutations can cause syndromes that increase the risk for atrial and/or ventricular arrhythmias. Cardiac channelopathies typically cause a “gain- or loss-of-function” in one of these currents by altering channel synthesis, channel transport to and from the cell surface membrane (trafficking), channel gating, and/or single channel permeation (Figure 2) (Delisle et al., 2004). This chapter provides a description for several different cardiac syndromes that can be caused by channelopathies and the utilization of the patch-clamp technique to learn about functional phenotypes.

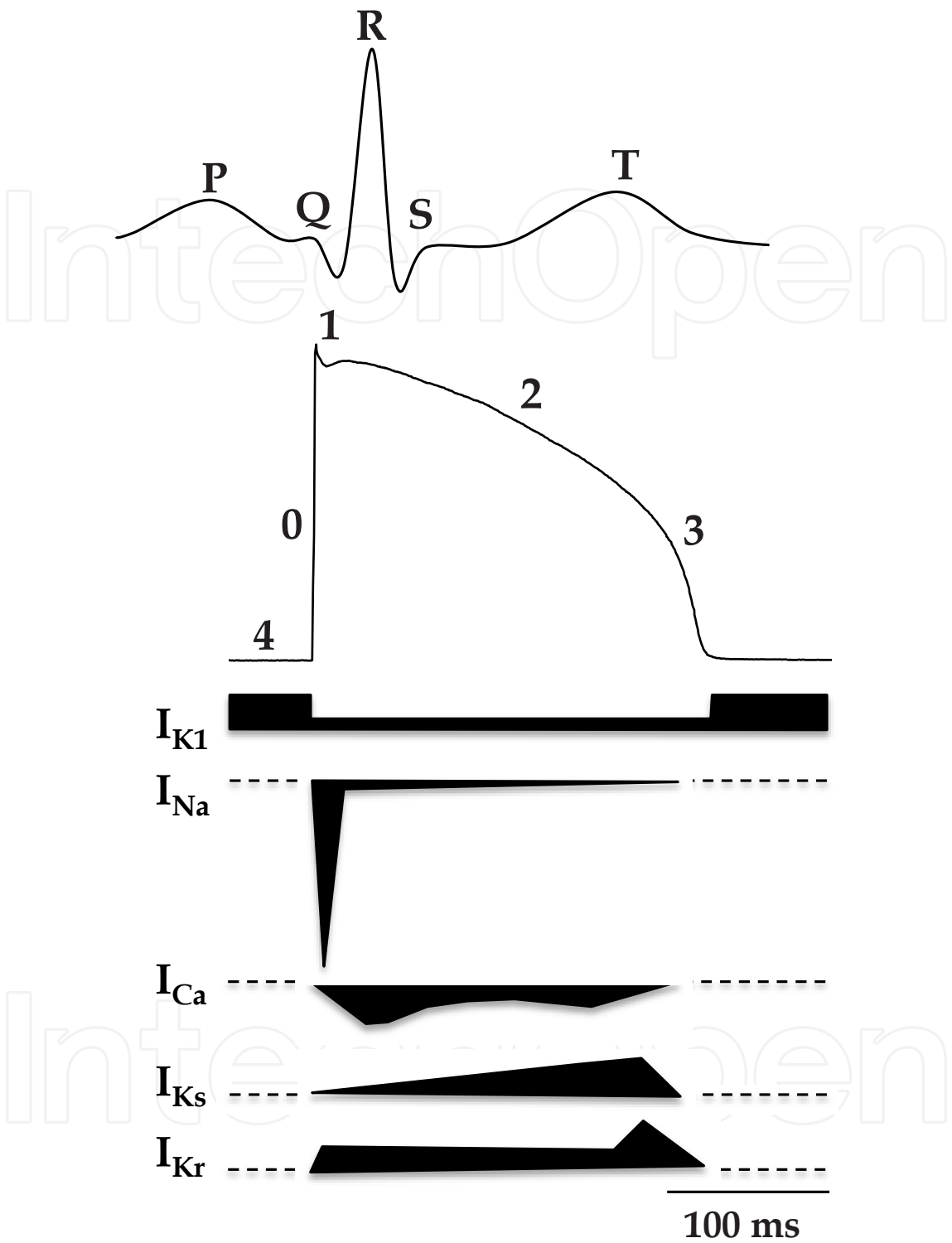


Fig. 1. The relationship between an ECG trace, a ventricular action potential, and important  $K^+$ ,  $Na^+$ , and  $Ca^{2+}$  currents. The P, Q, R, S, T correspond to the different waves on the ECG, the 4, 0, 1, 2, 3 correspond to the different phases of the ventricular action potential.  $I_{K1}$  is the inwardly rectifying  $K^+$  current,  $I_{Na}$  represents the  $Na^+$  current,  $I_{Ca}$  represents the  $Ca^{2+}$  current,  $I_{Ks}$  is the slowly activating delayed rectifier  $K^+$  current, and  $I_{Kr}$  is the rapidly activating delayed rectifier  $K^+$  current.

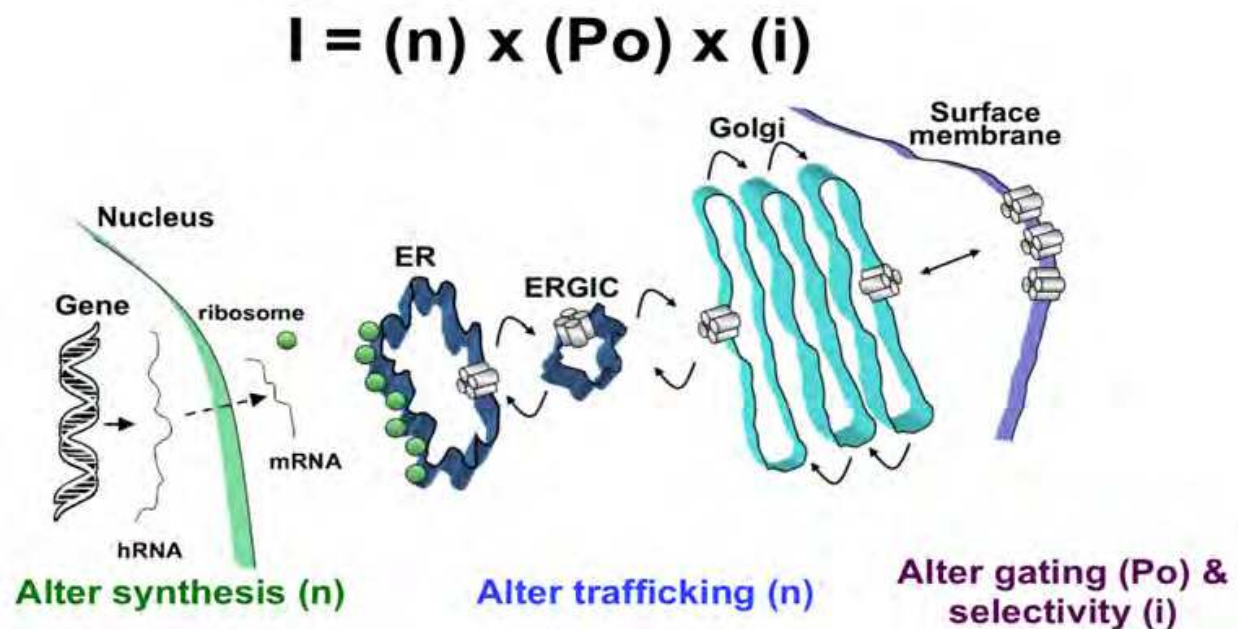


Fig. 2. A cartoon showing the synthesis, trafficking, and surface expression of ion channels. Macroscopic current ( $I$ ) is a function of channel number at the cell surface ( $n$ ), open probability ( $P_o$ ), and the amplitude of the single channel current ( $i$ ). Cardiac channelopathy mutations can alter  $n$  by changing synthesis and trafficking, or they can alter channel function by changing gating and selectivity (hRNA = heterogeneous ribonucleic acid; mRNA = messenger ribonucleic acid; ER = Endoplasmic Reticulum; ERGIC = ER Golgi Intermediate Compartment).

## 2. Cardiac channelopathies

Hundreds of different mutations in over a dozen different genes that encode cardiac  $K^+$ ,  $Na^+$  and  $Ca^{2+}$  channels or their regulatory proteins are linked to several different congenital arrhythmia syndromes including Long QT syndrome, Short QT syndrome, Brugada Syndrome, and familial Atrial Fibrillation (Lehnart et al., 2007).

### 2.1 Long QT syndrome

Congenital Long QT syndrome (LQT) is typically characterized by an abnormally long corrected QT (QTc) interval on an ECG. It is one of the most common “monogenic” arrhythmia syndromes and occurs in ~1:2,500 healthy births (Lehnart et al., 2007; Crotti et al., 2008). LQT-linked mutations are postulated to cause a prolongation of the plateau phase of the ventricular action potential. This allows L-type  $Ca^{2+}$  channels to recover from inactivation and reopen, which leads to an early after depolarization. Early after depolarizations increase risk for polymorphic ventricular tachycardia (torsade de pointes), which can cause a loss of cardiac output, syncope, and sudden death. The majority of LQT-linked mutations follow a dominant inheritance pattern (Romano-Ward type of LQT), and it is linked to at least thirteen different genes (LQT1-LQT13; Table 1) (Delisle et al., 2004; Ruan et al., 2008); however, autosomal-recessive forms, Jervell and Lange-Nielsen syndrome (JLN) exist and are caused by homozygous or compound mutations in *KCNQ1* and *KCNE1*. Most LQT-linked mutations cause a prolongation of the ventricular action potential

duration by either decreasing cardiac K<sup>+</sup> currents, or increasing the “late” Na<sup>+</sup> or Ca<sup>2+</sup> current. LQT1-3 account for 70-75% of all LQT cases, and the other 9 forms contribute an additional 5% (Kapa et al., 2009). The types of LQT mutations include splice, nonsense, frameshift, deletions, and missense, the latter type represents ~70% of the case-linked mutations.

Disease	Current	Chromosome	Defective gene (protein)	Frequency	Key reference
LQT1	I <sub>Ks</sub> , ↓ amplitude	11p15.5	KCNQ1 (Kv7.1)	~30-35%	Wang, et al., 1996
LQT2	I <sub>Kr</sub> , ↓ amplitude	7q35-q36	KCNH2, hERG (Kv11.1)	~30-35%	Curran et al., 1995
LQT3	I <sub>Na</sub> , ↑ late amplitude	3p22.2	SCN5a (Nav1.5)	~7-10%	Wang et al., 1995
LQT4	cell Ca <sup>2+</sup>	4q25-4q26	ANKB	Rare	Schott et al., 1995, Mohler et al., 2003
LQT5	I <sub>Ks</sub> , ↓ amplitude	21q22.1-22.2	KCNE1 (MiNK1)	Rare	Splawski et al., 1997
LQT6	I <sub>Kr</sub> , ↓ amplitude	21q22.11	KCNE2 (MiRP1)	Rare	Abbott et al., 1999
LQT7	I <sub>K1</sub> , ↓ amplitude	17q23-17q24.2	KCNJ2 (Kir2.1)	Rare	Tristani-Firouzi et al., 2002; Andelfinger et al., 2002.
LQT8	I <sub>Ca</sub> , ↑ amplitude	12p13.33	CACNA1C (Cav1.2)	Rare	Splawski et al., 2005
LQT9	I <sub>Na</sub> , ↑ late current	3p25	CAV3 (caveolin 3)	Rare	Vatta et al., 2006
LQT10	I <sub>Na</sub> , ↑ late current	11q23	SCN4B	Rare	Medeiros-Domingo et al., 2007
LQT11	I <sub>Ks</sub> , ↓ amplitude	7q21-q22	AKAP9 (Yotiao)	Rare	Chen et al., 2007
LQT12	I <sub>Na</sub> , ↑ late amplitude	20q11.2	SNTA1 (syntrophin-α1)	Rare	Ueda et al., 2008
LQT13	I <sub>KATP</sub> , ↓ amplitude	11q24.3	KCNJ5 (Kir3.4)	Rare	Yang et al., 2010
JLN1	I <sub>Ks</sub> , ↓ amplitude	11p15.5	KCNQ1 (Kv7.1)	Rare	Neyroud et al., 1997
JLN2	I <sub>Ks</sub> , ↓ amplitude	21q22.1-22.2	KCNE1 (MiNK1)	Rare	Schultze-Bahr et al., 1997

Table 1. Long QT syndrome disease types. Autosomal dominant forms (LQT1-12) include Romano Ward Syndrome (LQT1-6), Anderson Tawill Syndrome (LQT7), and Timothy Syndrome (LQT8). The autosomal recessive form is Jervell Lange-Neilson syndrome (JLN1-2) and is associated with deafness.

2.2 Short QT syndrome

Short QT syndrome (SQT) is characterized by a short QTc interval and can cause syncope, paroxysmal atrial fibrillation, and ventricular fibrillation (Lehnart et al., 2007; Patel et al.,

Disease	Current	Chromosome	Defective gene (protein)	Key reference
SQT1	I <sub>Kr</sub> , ↑ amplitude	7q35-q36	KCNH2, hERG (Kv11.1)	Brugada et al., 2004
SQT2	I <sub>Ks</sub> , ↑ amplitude	11p15.5	KCNQ1 (Kv7.1)	Bellocq et al., 2004
SQT3	I <sub>K1</sub> , ↑ amplitude	17q23-17q24.2	KCNJ2 (Kir2.1)	Hong et al., 2005
SQT4	I <sub>Ca</sub> , ↓ amplitude	12p13.33	CACNA1C (Cav1.2)	Priori et al., 2005
SQT5	I <sub>Ca</sub> , ↓ amplitude	10p12.33-p12.31	CACNB2 (Cavβ2)	Antzelevitch et al., 2007

Table 2. Short QT syndrome disease types.

2010). Similar to LQT, SQT follows a dominant inheritance pattern, but it is extremely rare and only a small number of families with missense mutations have been identified. SQT mutations are predicted to shorten the ventricular action potential duration by increasing cardiac K<sup>+</sup> currents or decreasing Ca<sup>2+</sup> current.

2.3 Brugada syndrome

Brugada syndrome (BrS) is characterized by ST segment elevation, conduction abnormalities (prolonged PR or QRS segments), and an increased risk of ventricular fibrillation (Lehnart et al., 2007). The prevalence of Brugada syndrome is slightly more common than LQT at ~1:2,000. The ECG changes are not always obvious but they can be usually seen after the administration of drugs that block the cardiac Na<sup>+</sup> current. The majority of BrS patients have missense, nonsense, deletion, or insertion mutations in *SCN5A* that cause a reduction in Na<sup>+</sup> current (Table 3). There appears to be much more genetic heterogeneity associates with Brugada syndrome, because ~2/3<sup>rds</sup> of the patients do not have a clear genotyped link. BrS-related cardiac events occur more often in adult men (this is likely because of gender differences in cardiac ion channel expression and hormone levels).

Disease	Current	Chromosome	Defective gene (protein)	Frequency	Key reference
BrS1	I <sub>Na<sub>v</sub></sub> ↓ amplitude	3p22.2	<i>ScN5a</i> (Nav1.5)	~10-30%	Chen, et al., 1998
BrS2	I <sub>Na<sub>v</sub></sub> ↓ amplitude	3p22.3	<i>GPDL1</i> (GPDL1)	Rare	London et al., 2007 Van Norstrand et al., 2007
BrS3	I <sub>Ca<sub>v</sub></sub> ↓ amplitude	12p13.33	<i>CACNA1C</i> (Cav1.2)	Rare	Antzelevitch et al., 2007
BrS4	I <sub>Ca<sub>v</sub></sub> ↓ amplitude	10p12.33-p12.31	<i>CACNB2</i> (Cavβ2)	Rare	Antzelevitch et al., 2007
BrS5	I <sub>Na<sub>v</sub></sub> ↓ amplitude	19q13.12	<i>SCN1B</i> (Navβ1)	Rare	Watanabe et al., 2008
BrS6	I <sub>TO</sub> , ↑ amplitude	11q13.4	<i>KCNE3</i> (MiRP2)	Rare	Delpon et al., 2008
BrS7	I <sub>Na<sub>v</sub></sub> ↓ amplitude	11q24.1	<i>SCN3B</i> (Navβ3)	Rare	Hu et al., 2009
BrS8	I <sub>F</sub> , ↑ amplitude	15q24.1	<i>HCN4</i> (HCN4)	Rare	Ueda et al., 2009
BrS9	I <sub>Ca<sub>v</sub></sub> ↓ amplitude	7q21.11	<i>CACNA2D1</i> (α2ζ)	Rare	Burashnikov et al., 2010

Table 3. Brugada syndrome disease types.

2.4 Familial Atrial Fibrillation

The most common arrhythmia is Atrial Fibrillation (AF), and it is usually secondary to structural changes in the heart (i.e. organ heart disease, valvular disease, hypertensive heart disease, cardiac hypertrophy, etc.). In some instances AF in the absence of structural heart disease (lone AF) occurs, and it is typically caused by genetic factors. Mutations in ion channel genes linked to familial forms of AF have been identified, but these mutations in these genes only account for a small number of cases (Lehnart et al., 2007).



Disease	Current	Chromosome	Defective gene (protein)	Key reference
ATFB3	$I_{Ks}$ , ↑amplitude	11p15.5	<i>KCNQ1</i> (Kv7.1)	Chen et al., 2003
ATFB4	$I_{Ks}$ , ↑amplitude	21q22.11	<i>KCNE2</i> (MiRP1)	Yang et al., 2004
ATFB7	$I_{Kur}$ , ↓amplitude	12p13.32	<i>KCNA5</i> (Kv1.5)	Olson et al., 2006 Yang et al., 2009
ATFB9	$I_{K1}$ , ↑□amplitude	17q24.3	<i>KCNJ2</i> (Kir2.1)	Xia et al., 2005
ATFB10	$I_{Na}$ , ↑ or ↓□amplitude	3p22.2	<i>SCN5a</i> (Nav1.5)	Darbar et al., 2008 Ellinor et al., 2008 Makiyama et al., 2008 Benito et al., 2008
ATFB11	$I_{CNX40}$ , ↓amplitude	1q21.2	<i>GJA5</i> (connexin 40)	Gollob et al., 2006
ATFB12	$I_{KATP}$ , ↓ADP current	12p12.1	<i>ABCC9</i> (Sur2A)	Olson et al., 2007

Table 4. Familial Atrial Fibrillation syndrome disease types linked to channelopathies.

2.5 Summary

Many different types of cardiac arrhythmias are linked to channelopathies. Thus far, mutations in over 20 different genes that encode cardiac ion channel  $\alpha$ -subunits, auxiliary channel subunits, or channel modulatory proteins are linked to one or more arrhythmia syndromes. This genetic heterogeneity has identified ion channel and macromolecular signaling complexes that are important for normal cardiac excitability and arrhythmia susceptibility. Many of these mutations are predicted to increase or decrease the macroscopic  $K^+$ ,  $Na^+$  or  $Ca^{2+}$  currents to increase arrhythmia susceptibility. This occurs by a number of different mechanisms, including alterations in protein synthesis, trafficking to/from the membrane, channel gating, and/or permeation.

3. Patch clamp techniques to study disease mechanisms

This section focuses on using the patch clamp technique to study disease mechanisms linked to the genetic variants in *KCNQ1* or *KCNH2*, which encode the voltage-gated  $K^+$  channel  $\alpha$ -subunits that underlie  $I_{Ks}$  (Kv7.1) and  $I_{Kr}$  (Kv11.1), respectively. Each Kv  $\alpha$ -subunit has six transmembrane (TM) segments that form a voltage-sensor (TM1-TM4) and a pore domain (TM5-TM6) (Figure 3).

Mutations in these genes are the most common cause of “monogenic” arrhythmia syndromes (Lehnart et al., 2007; Kapa et al., 2009). *KCNQ1* and *KCHN2* mutations can cause a loss- or gain-of-function (Wang et al., 1996; Neyroud et al., 1997; Chen et al., 2003). Loss of function mutations can be splice-site, nonsense, or frame-shift and result in haploinsufficiency; however, the vast majority of *KCNQ1* and *KCNH2* mutations linked to arrhythmia syndromes are missense. In syndromes that follow a dominant inheritance pattern (i.e. LQT, SQT, familial atrial fibrillation, etc) these mutant  $\alpha$ -subunits may co-assemble with wild type to generate functional phenotypes that cause a more severe phenotype (Figure 4).

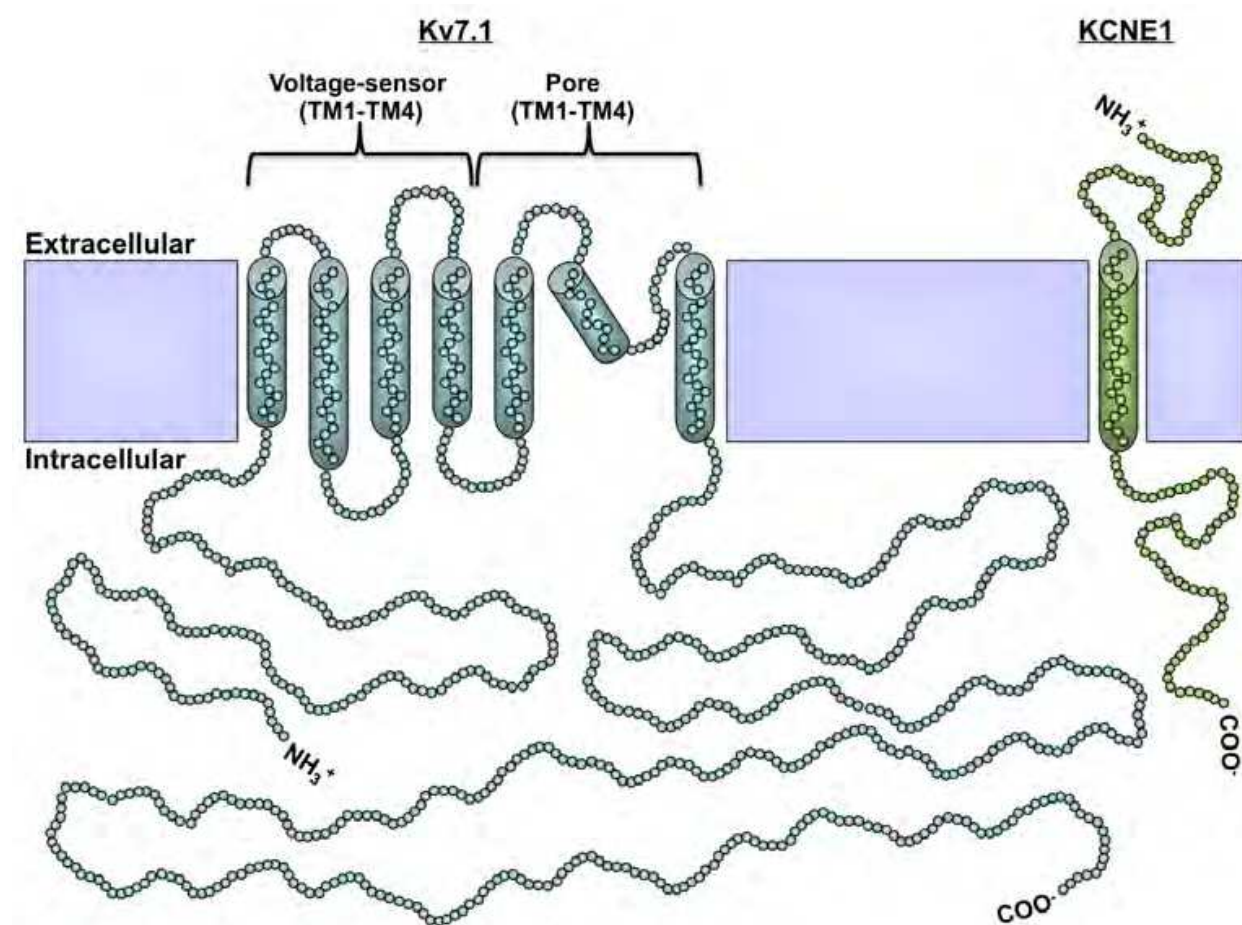


Fig. 3. A cartoon of a single Kv7.1  $\alpha$ -subunit and its putative auxiliary subunit KCNE1. The image shows a single Kv7.1  $\alpha$ -subunit and a single KCNE1 subunit. The membrane is represented by the purple rectangles, the individual amino acids are shown as small circles, and the secondary structures ( $\alpha$ -helices) are shown as cylinders. The respective amino and carboxy termini are labeled NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup>, respectively. Heterologous expression studies suggest that co-expression of Kv7.1 and KCNE1 are needed to generate macroscopic currents similar to native I<sub>Ks</sub> in the heart (Barhanin et al., 1996; Sanguinetti et al., 1996).

### 3.1 Long QT type 1

Thus far, hundreds of different mutations in *KCNQ1* have been linked to LQT1. Kv7.1 tetramerizes to form the aqueous channel of the I<sub>Ks</sub> in the heart (Kapa et al., 2009; <http://www.fsm.it/cardmoc/>). Mutations in *KCNQ1* account for ~30-35% of congenital LQT cases (Yuan et al., 2008; Kapa et al., 2009). Heterologous expression studies suggest that many LQT1 mutations throughout Kv7.1 cause a loss-of-function (Wollnik et al., 1997; Neyoud et al., 1998; Saarinen et al., 1998; Yamashita et al., 2001; Pippo et al., 2001; Deschênes et al., 2003; Yamaguchi et al., 2003; Thomas et al., 2005; Thomas et al., 2010).

LQT1 patients exhibit a wide-range of clinical phenotypes from asymptomatic to recurrent episodes of syncope and sudden cardiac arrest, and LQT1-related cardiac events are most often triggered by exercise (Priori et al., 2003; Napolitano et al., 2005; Schwartz et al., 2001; Priori et al., 2004; Ruan et al., 2008). Studies of multi-center LQT1 registries with hundreds



of patients suggest that the risk for LQT1 related cardiac events is related to the type/location of the LQT1 mutation (Zereba et al., 2003; Moss et al., 2007; Goldenberg et al.,

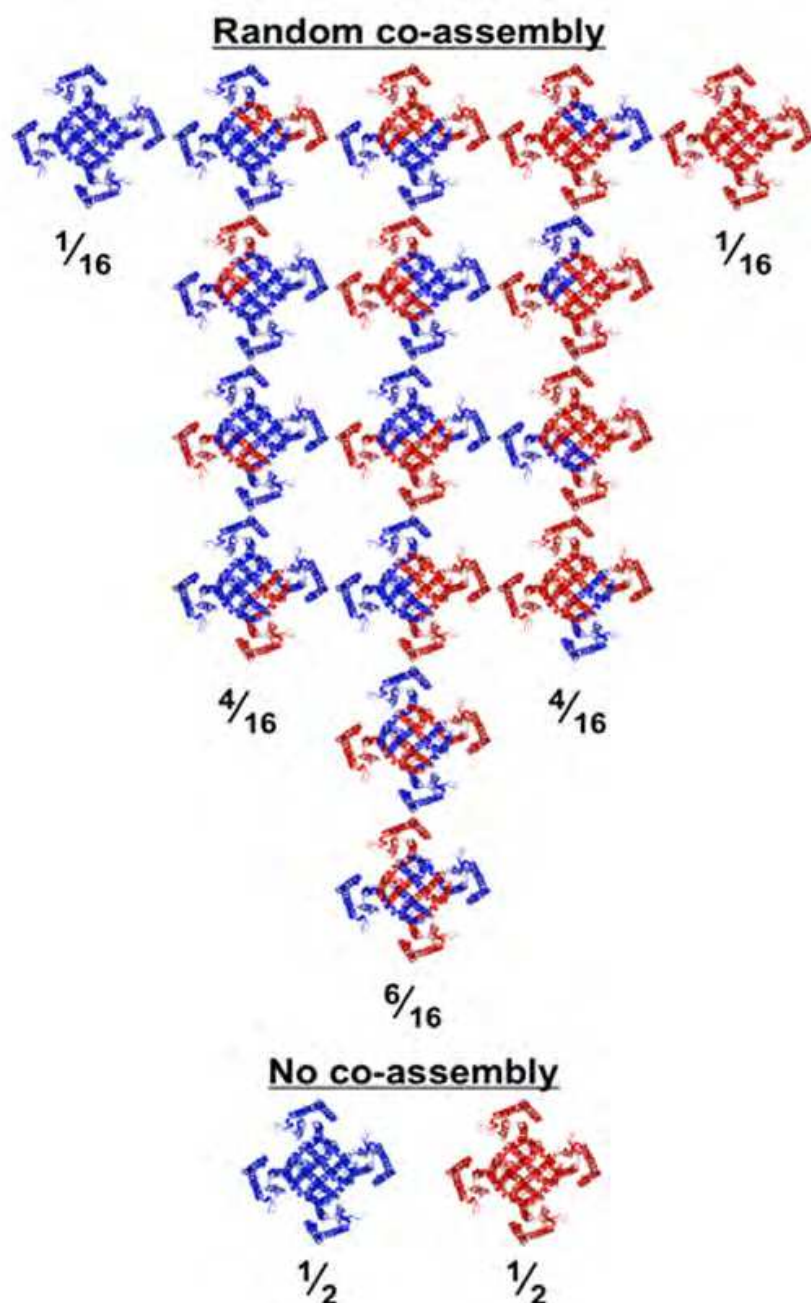


Fig. 4. A *cartoon* for two possible mechanisms of Kv  $\alpha$ -subunit co-assembly. The images show Kv tetramers from the “extracellular” view. The first scenario assumes that WT (blue) and mutant (red) Kv  $\alpha$ -subunits (blue) co-assemble both equally and randomly so only  $1/16^{\text{th}}$  of the total number of channels is all WT or mutant. The other  $14/16^{\text{ths}}$  of the channels are a combination of WT and mutant Kv  $\alpha$ -subunits with varying stoichiometries. If the WT and mutant Kv  $\alpha$ -subunits do not co-assemble, then half of the channels are all WT or mutant (January et al., 2000). The structural model for the Kv7.1  $\alpha$ -subunits used to generate the channels shown was developed by Smith and colleagues (2007).

2008; Jons et al., 2009). About 80% of LQT1 mutations are missense, and genetic testing for LQT1 is commercially available, but the diagnostic and prognostic value of a genotype positive test is limited because it does not distinguish between relatively benign or pathogenic variants (Kapa et al., 2009). Current treatments for LQT1 include life-style modification,  $\beta$ -blockers, implantable cardioverter defibrillators (ICDs), and/or left cardiac sympathetic denervation (Ruan et al., 2008). A major challenge for clinicians is to determine which therapeutic strategies are most appropriate for an individual. While many considerations need to be made, *in vitro* patch clamp studies of individual LQT1 mutations will provide a wealth of information, including identifying dominant negative functional phenotypes that may predict a more severe LQT1 clinical phenotype (Moss et al., 2007).

The most common way to determine if LQT1 mutations cause dominant negative suppression is to co-express WT and mutant channels in the heterologous expression systems. Initially a common heterologous expression system was *Xenopus laevis* oocytes. Indeed, the first studies that measured the functionality of LQT1-linked mutation utilized this system with the two-electrode voltage-clamp (Wang et al., 1999; Shalaby et al., 1997). An advantage to the *Xenopus* system is that investigators can control the amount of WT and mutant cRNA injected into cell. Unfortunately *Xenopus* oocytes express a Kv7 isoform that can make experiments with exogenously added Kv7.1/KCNE1 difficult to interpret (Lesage et al., 1993). Additionally, *Xenopus* oocytes are cultured at room temperature and the trafficking of many different LQT-linked mutations are temperature sensitive, which can also complicate the interpretation of the data (Zhou et al., 1999; Ficker et al., 2000; Anderson et al., 2006).

The whole-cell patch clamp technique of transiently expressing mammalian is an effective way to determine whether LQT1 mutations cause dominant negative effects. Mammalian cells can be transfected using chemical or electroporation techniques with Kv7.1 cDNA in plasmid expression vectors that contain WT-Kv7.1 or mutant Kv7.1 constructs. A disadvantage to this technique is that the uptake of the plasmid DNA is uncontrolled. Scenarios that further complicate this is that the cDNA for KCNE1 auxiliary channel subunits is also needed for transfection in order to generate native-like  $I_{Ks}$  (Barhanin et al., 1996; Sanguinetti et al., 1996). For co-expression studies that utilize multiple plasmids, WT-Kv7.1 and mutant cDNA plasmids are typically transfected in a 50:50 ratio (to mimic heterozygosity) using the total equivalent cDNA plasmid concentrations as in parallel experiments with WT or mutant only transfections. To favor the selection of cells that express multiple cDNA plasmids, cells are typically co-transfected with a fluorescent protein cDNA expression plasmid (i.e. green fluorescent proteins or GFP). The assumption is that cells expressing the fluorescent protein are likely to express the other plasmids as well.

Bartos and colleagues recently determined the effect of co-expressing WT-Kv7.1 and the LQT1-linked missense mutations E160K-Kv7.1 in Human Embryonic Kidney 293 (HEK293) (Bartos et al., 2011). This mutation has been previously characterized (Silva & Rudy, 2009; Wu D et al., 2010; Silva & Rudy, 2010;). The appropriate nucleotide change to generate the E160K-Kv7.1 mutations was engineered in the WT Kv7.1 (WT-Kv7.1) cDNA subcloned in pcDNA3 mammalian expression vector using the QuickChange Site Directed Mutagenesis

Kit (Stratagene, La Jolla, CA). HEK293 cells were transfected using Superfect (Qiagen Valencia, CA). Cells transfected with WT-Kv7.1 (3  $\mu$ g) plasmid DNA expressed small currents that did not resemble  $I_{Ks}$ , and so to recapitulate  $I_{Ks}$ -like currents, cells were also transfected with KCNE1 (3  $\mu$ g) plasmid DNA (total concentration was 6  $\mu$ g). The final ratio of KCNE1 to Kv7.1 cDNA ratio is  $\sim$ 2:1. For co-expression studies (to mimic heterozygosity), they transfected KCNE1 (3  $\mu$ g), WT-Kv7.1 (1.5  $\mu$ g), and E160K-Kv7.1 (1.5  $\mu$ g) plasmid DNA. Additionally, They transfected a third set of cells with KCNE1 (3  $\mu$ g) and E160K-Kv7.1 (3  $\mu$ g). Cells were also transfected with enhanced GFP cDNA subcloned in pKR5 expression plasmid (0.3  $\mu$ g). The cells were cultured in Minimum Essential Media (with 10% Fetal Bovine Serum) at 37 °C in 5% CO<sub>2</sub> and analyzed 24-30 hrs after transfection. Functional analyses were done using standard whole cell patch clamp technique on GFP positive cells. An Axopatch-200B patch clamp amplifier (Axon Instruments, Union City, CA) was used to measure macroscopic currents and cell capacitance. The uncompensated pipette resistances were 1-2 M $\Omega$  and series resistance was compensated between up to 95%. pCLAMP 10 software (Axon Instruments, Union City, CA) was used to acquire current signals and Origin (7.0, Microcal, Northhampton, MA) was used for performing data analyses. The external solution contained (in mM) 137 NaCl, 4 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 glucose, and 10 HEPES (pH 7.4 with NaOH), and an internal pipette solution contained (in mM) 130 KCl, 1 MgCl<sub>2</sub>, 5 EDTA, 5 MgATP, 10 HEPES (pH 7.2 with KOH). Figure 5 shows representative families of whole-cell currents measured from cells expressing KCNE1 with WT-Kv7.1, WT- and E160K-Kv7.1, E160K-Kv7.1. The currents were measured by applying “step” pulses from -80 mV to 70 mV in 10 mV increments for 5 s, followed by a “tail” pulse to -50 mV for 5 s. The intersweep holding potential and duration was -80 mV and 10 s, respectively. Cells expressing WT-Kv7.1 exhibited large macroscopic currents that resembled native  $I_{Ks}$ . Cells expressing WT- and E160K-Kv7.1 showed currents with similar characteristics as  $I_{Ks}$ , but the amplitudes were smaller than cells expressing WT-Kv7.1. Similar to that previously reported, cells expressing E160K-Kv7.1 did not generate hardly any current at all (Silva et al., 2009; Wu et al., 2010; Silva et al., 2010; Bartos et al., 2010). The mean peak tail current

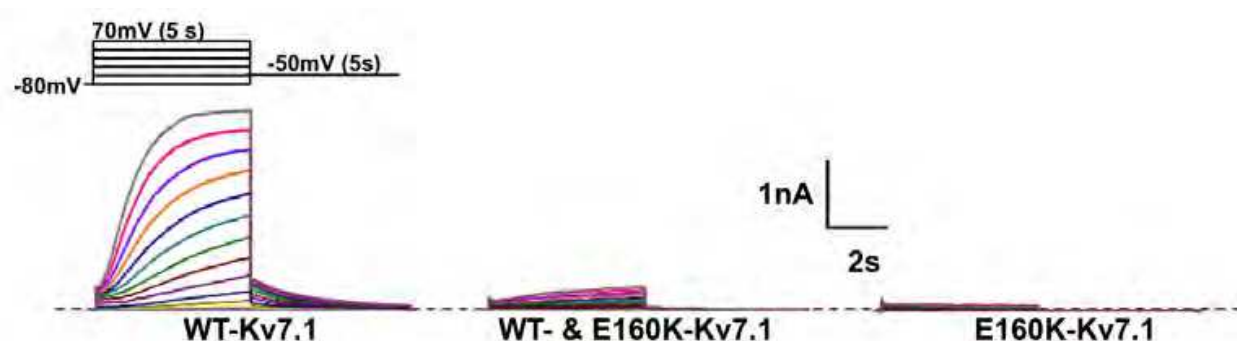


Fig. 5. The LQT1-linked mutation E160K-Kv7.1 causes dominant negative suppression of WT-Kv7.1. Representative families of whole-cell currents recorded from HEK293 cells expressing KCNE1 and WT-Kv7.1, WT- & E160K-Kv7.1, or E160K-Kv7.1. The corresponding voltage protocol used to measure the currents is also shown. Several mechanistic and computational studies suggest that E160K-Kv7.1 causes a loss of function by alter gating (Silva et al., 2009; Wu D et al., 2010; Silva et al., 2010; Bartos et al, 2011).

data for cells expressing WT-Kv7.1 or WT- and E160K-Kv7.1 were described using the Boltzmann function:

$$I = (I_{\text{MIN}} - I_{\text{MAX}}) / (1 + e^{(V - V_{1/2})/k}) + I_{\text{MAX}}$$

(1)

where  $I_{\text{MIN}}$  is the minimally activated current;  $I_{\text{MAX}}$  is the maximally activated current;  $V_{1/2}$  is the mid-point potential for half maximal activation; and  $k$  is the slope factor. Compared to cells expressing WT-Kv7.1, cells expressing WT- and E160K-Kv7.1 did not alter  $I_{\text{MIN}}$ ,  $V_{1/2}$ , or  $k$ , but it did reduce  $I_{\text{MAX}}$  by more than half that of cells expressing WT-Kv7.1. These data suggest that E160K-Kv7.1 causes dominant negative suppression of WT-Kv7.1. Additionally, this mutation is associated with a relatively severe LQT1-related clinical phenotype (Silvia et al., 2009).

3.2 Long QT type 2

Similar to LQT1, LQT2 is linked to hundred of different *KCNH2* mutations (Kapa et al., 2009; <http://www.fsm.it/cardmoc/>); mutations in *KCNH2* account for ~30-35% of congenital LQT cases (Yuan et al., 2008; Kapa et al., 2009); and heterologous expression studies suggest that many LQT2 mutations throughout the Kv11.1  $\alpha$ -subunit cause a loss-of-function (Sanguinetti et al., 1996; Zhou et al., 1998; Zhou et al., 1999; Chen et al., 1999; Furutani et al., 1999; Nakajima et al., 1999; Lees-Miller et al., 2000; Huang et al., 2001; Paulussen et al., 2002; Hayashi et al., 2002; Yang et al., 2002; Johnson et al., 2003; Hayashi et al., 2004; Rossenbacker

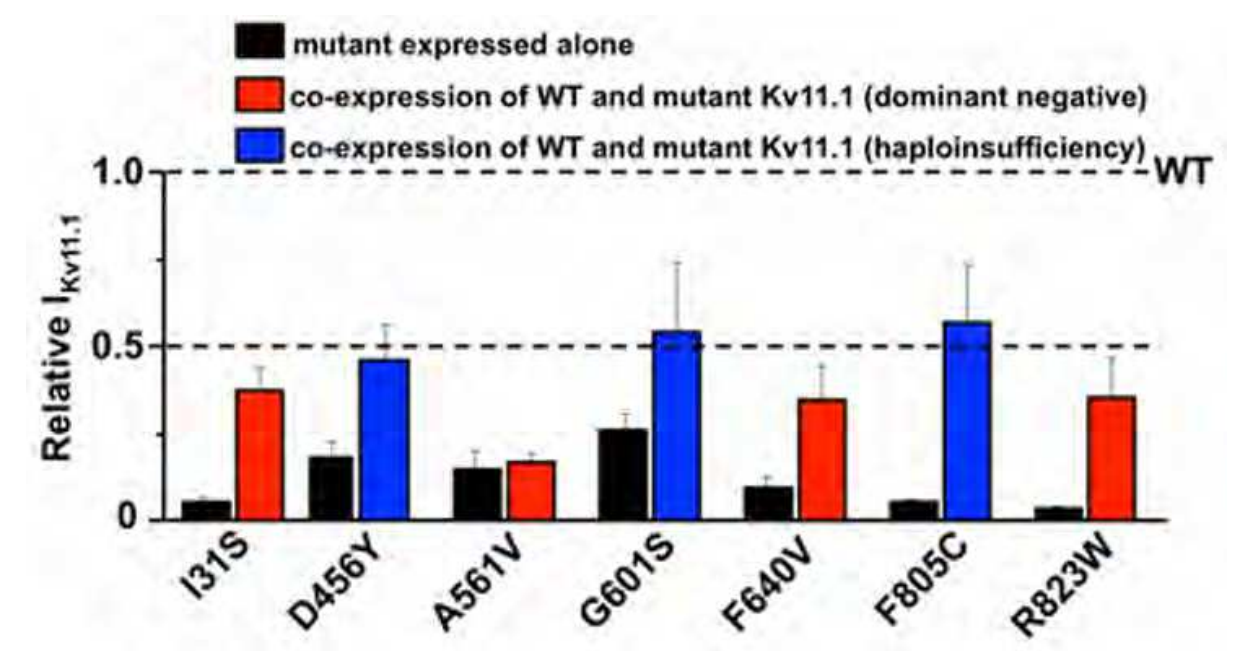


Fig. 6. Trafficking-deficient LQT2-linked mutations generate a spectrum of functional phenotypes. Maximally activated currents were measured from cells expressing WT-Kv11.1, mutant Kv11.1, or WT and mutant Kv11.1 (Modified from Anderson et al., 2006). The relative amplitudes compared to WT-Kv11.1 are shown. Mutations expressed alone all reduced maximally activated current by > 0.5 (black). Co-expression of WT and Kv11.1 mutations suggested that are caused dominant negative effects and reduced maximally activated current by >0.5 (red), whereas others resulted in haploinsufficiency and reduced maximally activated current by ~0.5 (blue).



et al., 2005; Anderson et al., 2006; Gong et al., 2007). Studies suggest that approximately 90% of LQT2 missense mutations cause a loss-of-function preventing the trafficking of the mutant Kv11.1 to the cell surface membrane (Anderson et al., 2006). These “trafficking-deficient” mutations can cause dominant negative effects by inhibiting the trafficking of WT-Kv11.1 (Ficker et al., 2000). Using similar techniques as described above co-expressing WT-Kv7.1 and LQT1 mutations, Anderson and colleagues (2006) co-expressed WT-Kv11.1 with different trafficking-deficient LQT2 mutations in HEK293 cells. Cells were depolarized to 50 mV for 5 seconds, and the maximally activated peak tail  $I_{Kv11.1}$  was measured at a test pulse to -120 mV for 3 seconds. Figure 6 shows the relative mean peak amplitude of tail current measured from cells expressing only the trafficking-deficient LQT2 mutations or co-expressing WT-Kv11.1. The data suggest some trafficking-deficient LQT2 mutations can cause dominant negative effects whereas others cause haploinsufficiency.

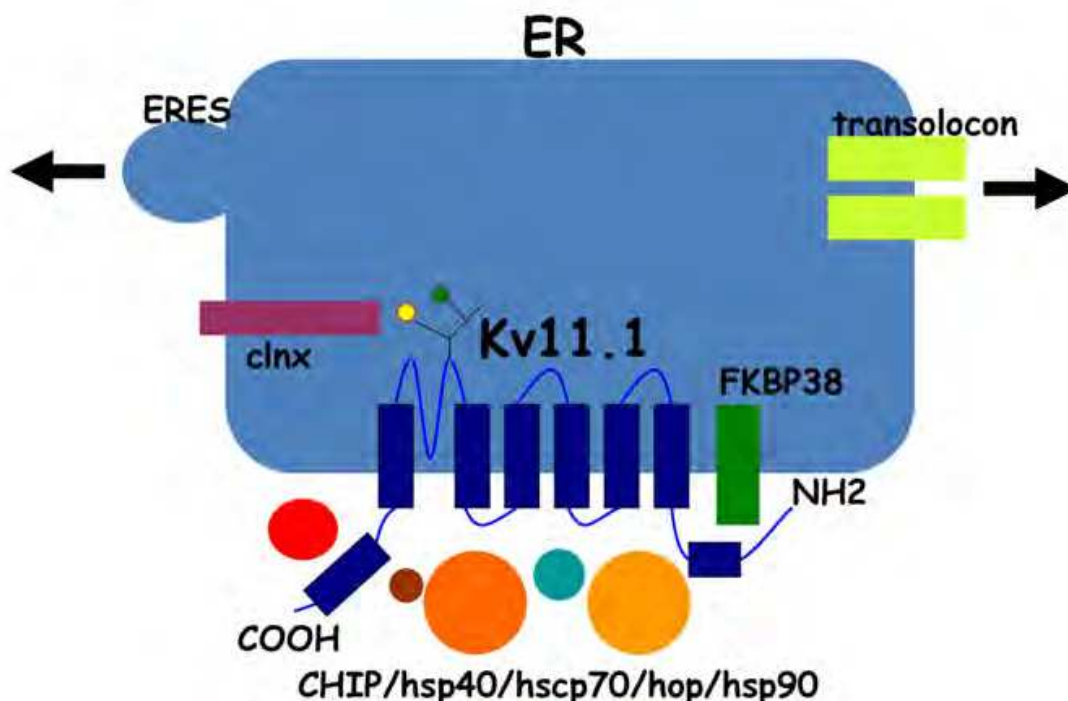


Fig. 7. A cartoon showing the different chaperones that regulate the trafficking of Kv11.1 in the secretory pathway. In the ER, several different chaperones and co-chaperones, including calnexin (clnX), CHIP, heat shock proteins 40, 70, 90 (hsp40, hsc70, hsp90), hop, and FKBP38 associate with Kv11.1. Misfolded Kv11.1 is prevented from trafficking into the secretory pathway in Endoplasmic Reticulum Exit Sites (ERES) and is degraded in the translocon/proteasomal pathway.

Trafficking-deficient LQT2 channels are retained in the Endoplasmic Reticulum (ER). Several studies suggest that the mutations cause “misfolding” of Kv11.1 and cellular quality control mechanisms prevent its export into the secretory pathway (Ficker 2003; Delisle et al., 2005; Gong et al., 2005; Gong et al., 2006; Walker et al., 2007; Walker et al., 2010; Li et al., 2010). Indeed, many different molecular chaperone proteins that regulate the trafficking of WT-Kv11.1 and LQT2 mutations have been identified (Figure 7). Misfolded Kv11.1 undergoes degradation through the proteasome pathway. To understand how



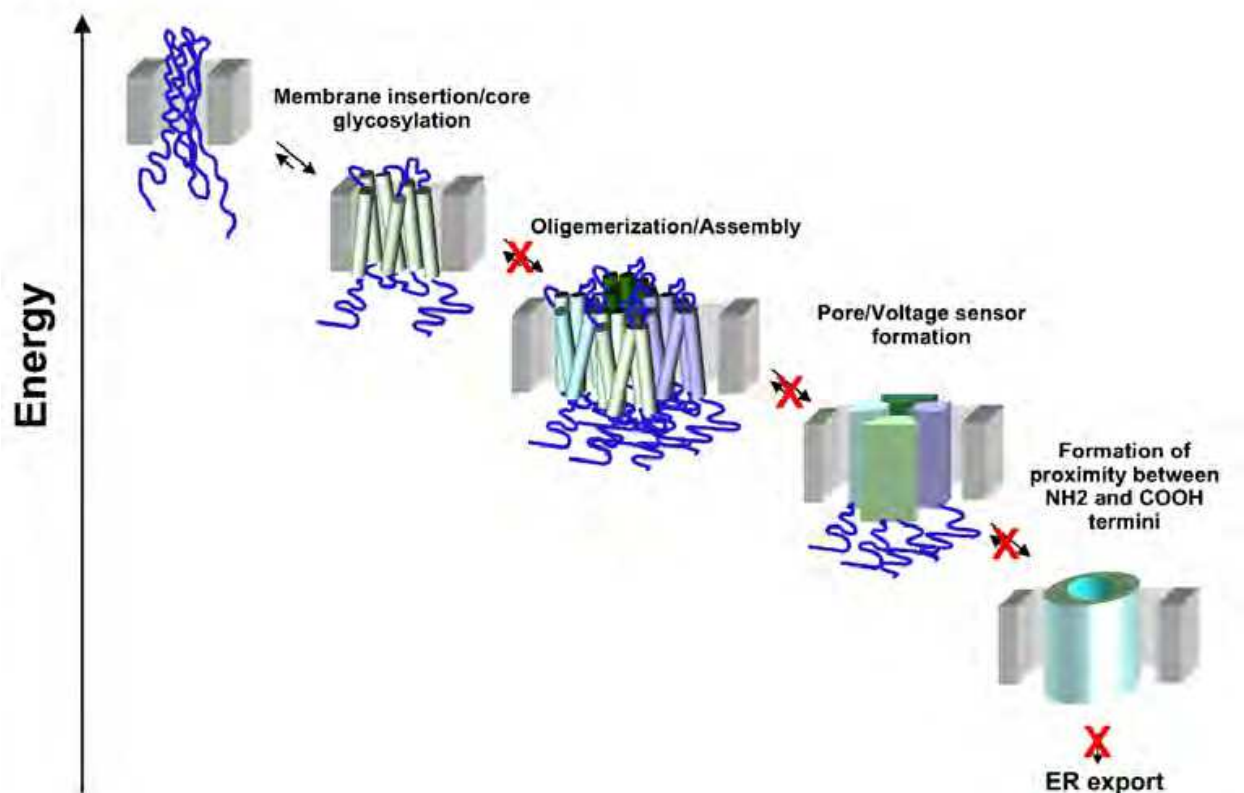


Fig. 8. A cartoon showing how some trafficking-deficient LQT2 mutations may disrupt distinct steps in Kv11.1 biogenesis. Trafficking-deficient LQT2 mutations may cause haploinsufficiency by preventing the oligomerization or co-assembly of WT- and mutant Kv11.1  $\alpha$ -subunits. Alternatively, mutant Kv11.1  $\alpha$ -subunits may cause dominant negative effects by co-assembling with WT-Kv11.1 and disrupt pore/voltage sensor formation or formation of proximity between the amino (NH<sub>2</sub>) or carboxy (COOH) termini. The cartoon shows the different stages of biogenesis, from the formation of secondary structures (cylinders) to the a folded channel (large cylinder with a circle).

trafficking-deficient LQT2 mutations may cause dominant negative or haploinsufficiency, the steps that underlie the assembly and folding of Kv channel proteins during biogenesis need to be understood. Schulteis and colleagues (1998) proposed the following sequence of events during Shaker K<sup>+</sup> channel biogenesis in the ER: 1) membrane insertion and core-glycosylation, 2) assembly of Shaker  $\alpha$  and  $\beta$  subunit channel proteins, 3) formation of the pore and voltage sensor, and 4) formation of proximity between adjacent amino and carboxy termini. Assuming a similar sequence of events for Kv11.1 (Figure 8), then trafficking-deficient LQT2 mutations may be capable of disrupting different steps in Kv11.1 channel biogenesis to cause either dominant negative or haploinsufficiency. LQT2 mutations that do not co-assemble do not cause dominant negative suppression of WT-Kv11.1 channel proteins (Ficker et al., 2002) and this would suggest that some trafficking-deficient LQT2 mutations disrupt Kv11.1 biogenesis before the co-assembly step(s). Dominant negative trafficking-deficient LQT2 disrupt Kv11.1 biogenesis steps after co-assembly. The latter group may disrupt Kv11.1 biogenesis steps such as the folding of the voltage sensor/pore, or the formation of proximity between adjacent amino and carboxy termini.

### 3.3 Summary

The most common cardiac channelopathies are caused by mutations in *KCNQ1* (Kv7.1) and *KCNH2* (Kv11.1) that follow a dominant inheritance pattern and cause LQT1 and LQT2, respectively. The vast majority of these mutations are missense, and they can co-assemble with WT  $\alpha$ -subunits to cause effects that range from less than or more than (dominant negative) haploinsufficiency. *In vitro* techniques that utilize the whole cell patch clamp can identify dominant negative mutations that suppress channel function and/or trafficking. We conclude that the patch clamp technique is useful for identifying particularly pathogenic LQT1 and LQT2 mutations.

### 4. Conclusions

Cardiac channelopathies have informed us about the channels and proteins that are important for atrial and ventricular excitability. Correlations between *in vitro* and clinical data suggest that patch clamp technique can provide prognostic information about individual mutations. We expect advancements in genotyping and high throughput molecular, tissue culture, and patch clamp techniques may enable clinicians to assess the risk of individual patient mutations identified through genotyping using *in vitro* patch clamp assays.

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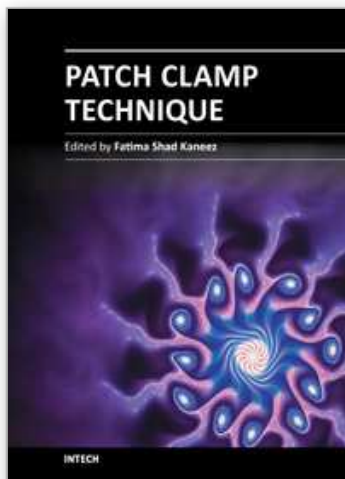
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### **Patch Clamp Technique**

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This book is a stimulating and interesting addition to the collected works on Patch clamp technique. Patch Clamping is an electrophysiological technique, which measures the electric current generated by a living cell, due to the movement of ions through the protein channels present in the cell membrane. The technique was developed by two German scientists, Erwin Neher and Bert Sakmann, who received the Nobel Prize in 1991 in Physiology for this innovative work. Patch clamp technique is used for measuring drug effect against a series of diseases and to find out the mechanism of diseases in animals and plants. It is also most useful in finding out the structure function activities of compounds and drugs, and most leading pharmaceutical companies used this technique for their drugs before bringing them for clinical trial. This book deals with the understanding of endogenous mechanisms of cells and their receptors as well as advantages of using this technique. It covers the basic principles and preparation types and also deals with the latest developments in the traditional patch clamp technique. Some chapters in this book take the technique to a next level of modulation and novel approach. This book will be of good value for students of physiology, neuroscience, cell biology and biophysics.

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